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Mercury in Fish in North Mississippi Reservoirs: Statistical Analysis and Risk Assessment

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MERCURY IN FISH IN NORTH MISSISSIPPI RESERVOIRS: STATISTICAL ANALYSIS AND RISK ASSESSMENT

by
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ABSTRACT

Mercury (Hg) is a pollutant of particular concern because it is wide-spread, persistent, and poses a serious risk to human health. Once released into the atmosphere, it cycles through the environment by a series of complex biogeochemical processes. Upon deposition to aquatic ecosystems, inorganic mercury can be transformed to methylmercury by microorganisms. Methylmercury is a known human neurotoxin that can bioacculmulate in fish tissue and biomagnify up aquatic food chains. Consumption of contaminated fish is the primary route of human exposure to methylmercury.

The Mississippi Department of Health has issued fish consumption advisories for Grenada and Enid Lakes in the Yazoo River Basin as a result of elevated mercury concentrations. This study involved a statistical analysis of mercury data for Crappie (CR), Largemouth Bass (LMB), and Channel Catfish (CC) from Grenada, Sardis, and Enid Lakes in Northern Mississippi; total Hg concentrations were compared between Lakes and between species. A mercury risk assessment for consumption of fish from the lakes was also conducted using different assumption variables to evaluate the effectiveness of the existing fish consumption advisories.

Linear regression analysis of length vs. weight suggested that LMB and CC exhibited similar growth trends regardless of lake. The relationship between length and weight for CR from Enid Lake was statistically different from that of CR from Grenada and Sardis, suggesting that environmental factors unique to Enid Lake may affect the growth of CR there. Of the fish analyzed, LMB consistently had the highest mean

mercury concentrations (mean ± SE, n = 14 – 20, Grenada: 630 ± 104 ng/g, Sardis: 334 ± 40 ng/g, Enid: 386 ± 36 ng/g), followed by CC (Grenada: 437 ± 42 ng/g, Sardis: 222 ± 21 ng/g, Enid: 152 ± 14 ng/g) and then CR (Grenada: 199 ± 17, Sardis: 147 ± 8 ng/g, Enid: 214 ± 10 ng/g). Even taking length into account, Grenada Lake produced the LMB and CC with the highest Hg concentrations, suggesting the Hg concentration may be higher there than at Sardis and Enid.

Only LMB had a strong relationship between length and Hg concentration by linear regression analysis. Because the existing fish consumption advisories are length-based, the lack of relationship between length and Hg concentration means the recommendations may be insufficient to protect the public from exposure to MeHg.

Seven different risk assessment paradigms yielded hazard index (HI) and monthly consumption limit (MCL) values that further discredit the existing consumption advisories and many consumption recommendations. An HI greater than one is indicative of an individual's risk of toxicity associated with exposure to a toxicant, here methylmercury. LMB from Grenada had an adult HI>1 by all seven risk calculations. Similarly, all fish species from all three lakes yielded HI>1 for children.

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ABBREVIATIONS

ADCNR	Alabama Department of Conservation and Natural Resources
AHA	American Heart Asssociation
CC	Channel Catfish (*Ictalurus punctatus*)
CR	Crappie (*Pomoxis annularis*)
EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Administration
LMB	Largemouth Bass (*Micropterus salmoides*)
MDEQ	Mississippi Department of Environmental Quality
MDWFP	Mississippi Department of Wildlife, Fisheries, and Parks
MeHg	Methylmercury (CH_3Hg)
MMRI	Mississippi Minerals Resources Institute
NOAA	National Oceanic and Atmospheric Administration
TWPD	Texas Wildlife and Parks Department
UNEP	United Nations Environment Programme
USGS	United States Geological Survey

I. INTRODUCTION AND BACKGROUND

1.1 MERCURY IN THE ENVIRONMENT

1.1.1 Sources of mercury

Mercury (Hg) is present in the environment in three forms that are important to human health: elemental mercury (Hg^0), gaseous oxidized mercury (Hg^{2+}), and organometallic methylmercury (CH_3Hg). Each form has unique physical and chemical properties that dictate its behavior in the environment. Mercury cycles between its various species by a series of complex biogeochmical processes (UGSG 2013). Once released into the environment, mercury can travel far from its original source, making it a global concern.

In the atmosphere, mercury exists mostly as a stable, monoatomic gas (Hg^0). Hg^0 is released as a result of natural processes such as evaporation from soil and water or volcanic emissions. Hg^0 is also released from anthropogenic sources such as coal-burning power plants, precious metal extraction, industry, and mining (Clarkson et al 2003; Driscoll et al 2013). Gaseous oxidized Hg^{2+} is by-product of forest fires and other natural combustion processes (MDEQ 2002). Human activity that disturbs land and releases Hg also contributes to environmental concentrations (Chalmers et al 2011). Because anthropogenic sources emit more Hg than natural sources, the concentration of mercury circulating in the environment has greatly increased in the last century (Driscoll et al 2013; Shimshack et al 2007). Recent estimates of global mercury emissions are 6500 to 8200 Mg/year, and nearly 70% of those emissions are attributed to anthropogenic sources (4600 to 5300 Mg/year) (Driscoll et al 2013).

Sources of mercury can be classified as primary or secondary. Primary sources include industrial processes that produce mercury as a by-product and natural processes such as weathering of mercury-containing rocks or volcanic emissions. Secondary sources, such as burning biomass and recycling mercury-containing products, merely redistribute existing environmental mercury within and between ecosystems (Driscoll et al 2013; UNEP 2008). Both natural and anthropogenic primary sources release Hg into the atmosphere, increasing the global concentration of mercury cycling through the environment.

1.1.2 Biogeochemical cycling of mercury

Elemental mercury vapor is weakly soluble and must be transformed through photochemical oxidation mechanisms to water-soluble, inorganic Hg^{2+} before it appreciably enters aquatic environments (MDEQ 2002). Inorganic mercury can be transferred to aquatic systems via deposition from the atmosphere, primarily associated with rainwater runoff (USGS 2013). Once Hg^{2+} enters aquatic ecosystems it can move between biospheres (for example, water to sediment) and enter the food chain. Alternatively, Hg^{2+} can be reduced back to Hg^0 by microorganisms and return to the atmosphere via volatilization (Clarkson et al 2003; USGS 2013).

The atmosphere is the primary transportation route for gaseous mercury species. Natural land and ocean processes are responsible for both the movement of mercury in terrestrial and aquatic ecosystems and the production of methylmercury

(CH_3Hg) (Driscoll et al 2013). Thus, mercury can cycle through the environment for extended periods of time (Fig. 1).

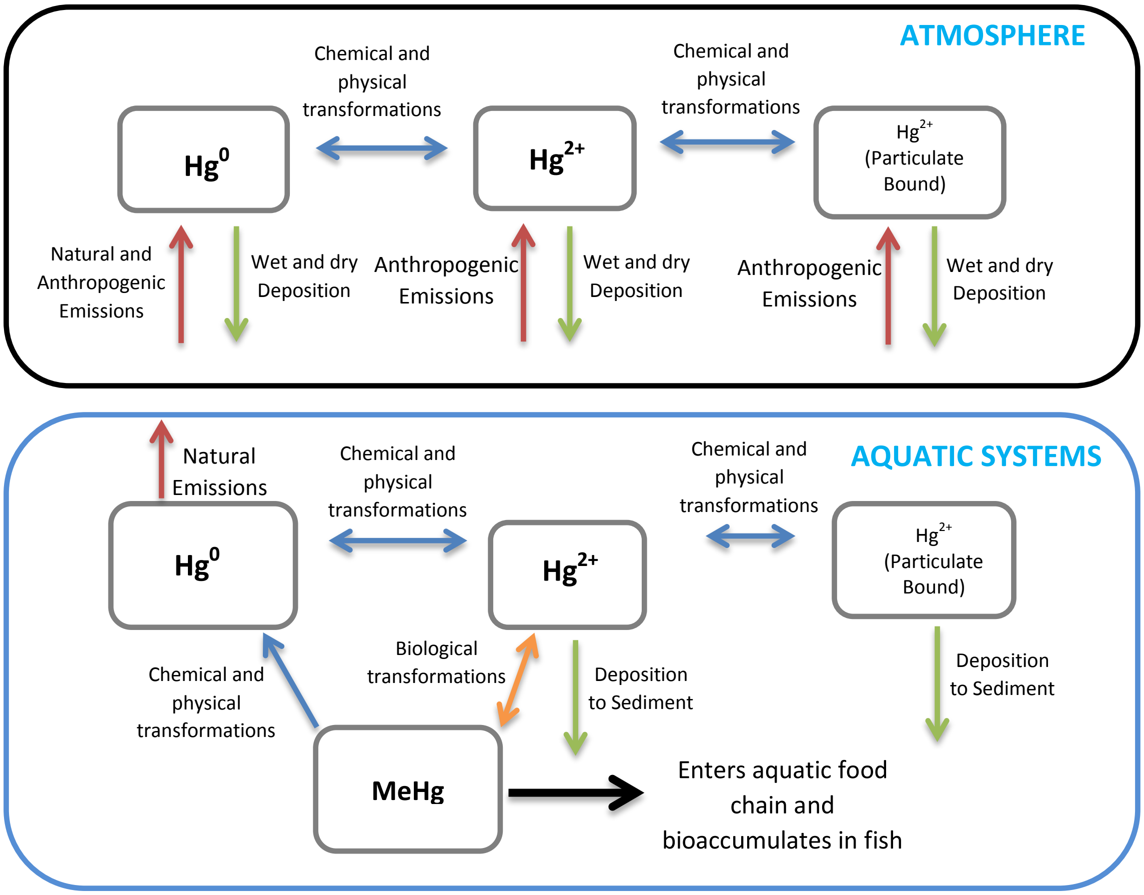

Figure 1: Biogeochemical pathways by which mercury cycles through its various forms in the environment.

1.2 METHYLMERCURY

1.2.1 Methylation and demythelation processes

Anaerobic microorganisms in reducing zones of freshwater and coastal ecosystems are responsible for the methylation of Hg^{2+} present in sediment to form methymercury (MeHg). Microorganisms can also facilitate the demethylation of MeHg back to inorganic Hg^{2+} and the subsequent reduction to Hg^0 (MDEQ 2002). The methylation and demethylation processes are largely executed by sulfate-reducing bacteria (MDEQ 2002). Iron reducing mircoorganisms also play a role in the transformations, but to a lesser extent (Driscoll et al 2013).

MeHg formation is governed by many factors including sulfate concentrations, Hg^{2+} availability, and the activity of sulfate-reducing bacteria. At very high sulfate concentrations, SO_4^{2-} binds to the Hg^{2+} in sediment, rendering it unavailable for methylation. Methylation often takes place in sediment as a result of the Hg^{2+} that accumulates there as well as the anaerobic conditions required for the reduction reactions (MDEQ 2002). Water conditions that affect the formation of MeHg include depth, temperature, dissolved oxygen content, total dissolved solids, and pH (MMRI 2007). Methylation of Hg^{2+} is most prevalent in acidic ecosystems with higher concentrations of organic material. Dissolved organic matter acts as an Hg "carrier," binding to Hg^{2+} and altering its bioavailability (Driscoll et al 2013).

Fish are exposed to MeHg through their diet, the water, and the sediment. Bioaccumulation refers to the buildup of MeHg in the tissue of an organism. MeHg is also subject to biomagnification, a phenomenon in which a toxin is found at increased concentrations in the tissues of organisms at higher trophic levels; therefore, MeHg concentrations are typically highest in the muscle tissue of larger, long-living predatory fish. The long half-life of MeHg in fish (1-3 years) contributes to its bioaccumulation to such high levels in aquatic food chains (MDEQ 2002). Ultimately, the concentration of mercury in fish tissue depends on how much mercury the fish are exposed to in their diet and how well they can metabolize and then excrete it. Fish tend to accumulate more mercury with increasing size and age due to longer exposure times, slower elimination rates, and higher trophic levels (Chalmers, et al 2003; Driscoll et al 2013).

1.2.2 Health risks of methylmercury exposure

Mercury is a pollutant of particularly great concern because of the risks to human health associated with exposure. It is a non-essential metal known for its neurotoxicity, especially in infants and children (EPA 2013). Methylmercury is the most toxic form of mercury in the environment, and therefore, warrants the most attention (USGS 2013). The Center for Disease Control (CDC) found that one in ten women of childbearing age has elevated mercury concentrations which can pose a risk of neurological damage during fetal development (Shimshack et al 2007).

Concentrations of MeHg in ambient air and water are largely considered too low to pose a serious threat to human health (Clarkson et al 2003). According to the United States Environmental Protection Agency (US EPA), consumption of contaminated fish is the sole source of human exposure to MeHg. Typical preparatory and cooking methods do very little to lessen the exposure to MeHg (Shimshack et al 2007). Once consumed approximately 95% of the MeHg is absorbed into the bloodstream (Huggett et al 2001). Consumption of MeHg results in neurotoxicity, even at seemingly low concentrations, if exposure persists over long periods of time (EPA 2013). Because MeHg can cross both the placental and blood-brain barriers, exposure during prenatal and childhood development is of especially great concern (UNEP 2008). Higher concentrations consumed in short time spans pose other health risks, including kidney damage, cardiovascular collapse, and death (Hugget et al 2001).

1.3 MERCURY IN MISSISSIPPI

The Mississippi Department of Environmental Quality (MDEQ) issues fishing bans and consumption advisories for contaminated water bodies in an attempt to help protect people who regularly consume fish caught in areas that may be impaired by mercury or other toxins. As of April 2012, eleven water bodies (Fig. 2), including the Gulf of Mexico are under consumption advisories for mercury (MDEQ 2012). Among those listed are Enid Reservoir and Grenada Lake, which both have consumption limits for both largemouth bass and catfish exceeding 27 inches in length and for king mackerel in the Gulf. The advisories were issued based on the high concentrations of mercury in fish tissues sampled by the MDEQ in 1996. Enid Reservoir has been under a consumption advisory since May 1995 and Grenada since June 2001 (MDEQ 2007). Though Sardis Lake also has relatively high mercury levels, the Mississippi Department of Health has not issued a consumption advisory yet (Huggett et al 2001; MDEQ 2007). The Mississippi guideline for total mercury concentrations in fish is 0.75 ppm and the federal limit is 1.00 ppm. Largemouth bass collected from Enid Reservoir in 1996 had a mean concentration of 1.07 ppm. Fish collected from Grenada and Sardis Lakes during that same study had concentrations of 1.07 ppm and 0.85 ppm, respectively (Huggett et al 2001).

According to the Mississippi Department of Health, the source of mercury that is contaminating lakes in Northern Mississippi is unknown. There are no industries in the northern region of the state that release enough mercury to cause the elevated concentrations being found (MDEQ 2007). The MDEQ claims it is committing a large portion of its resources to investigating possible sources of mercury contamination and to monitoring the status of Mississippi water bodies currently impaired due to Hg. Additionally, due to new data suggesting that mercury can be harmful to human health at concentrations much lower than previously stated, the MDEQ has placed a new focus on fish species that have lower tissue concentrations (MDEQ 2012).

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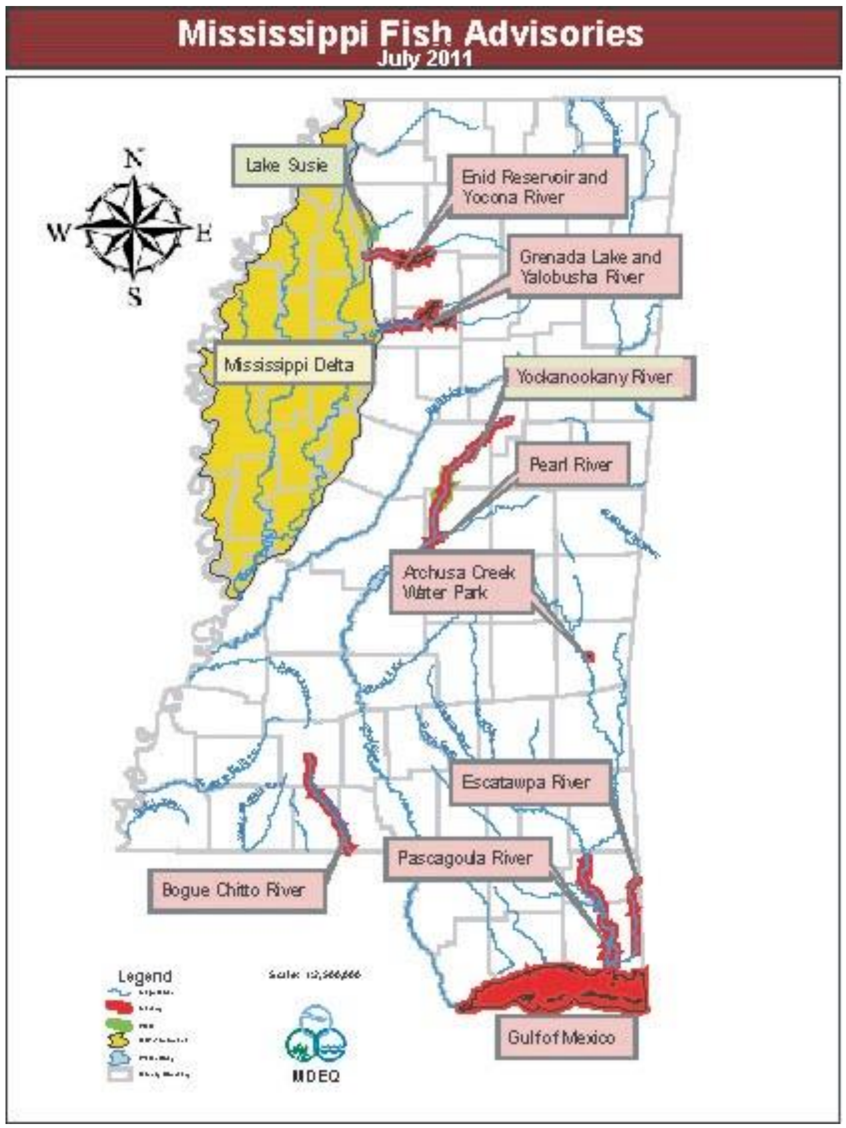


Figure 2: Map of fish consumption advisories in Mississippi (MDEQ 2012)

1.4 HISTORY OF CONSUMPTION ADVISORIES

Mercury has not always been a pollutant of major concern for governmental agencies. The 1940s through the 1970s saw rapid industrial growth and inadequate water treatment, creating many point sources of Hg in urban areas. As environmental mercury concentrations increased, there was a growing concern for the risks of exposure to human health. In the 1970s the Great Lakes region became the first to issue local fish consumption advisories to protect people from the rising Hg concentrations in the area's lakes and rivers (Lando & Zhang 2011). However, most government agencies discounted the concern about rising mercury emissions. Even as late as 1994, the US Food and Drug Administration (FDA) still maintained that mercury toxicity was not a risk with "normal patterns of consumption" (Shimshack et al 2007).

The FDA reconsidered its stance on the risks of methylmercury consumption in 2000 and issued its first national fish consumption advisory in early 2001 (Shimshack et al 2007). The new policy was a direct response to reports released by the EPA and the National Academy of Science (NAS) which outlined the health risks associated with exposure to mercury from contaminated fish. The FDA's advisory targeted "at risk" consumers, including women who were pregnant or nursing and young children, advising them against eating the species of fish with the highest mercury concentrations: shark, swordfish, king mackerel, and tilefish (Lando & Zhang 2001; Shimshack et al 2007). In 2004 the FDA and the EPA joined forces to reissue the advisory, expanding it to encompass canned fish and including more details about recreationally caught fish species (Lando & Zhang 2011). The new joint advisory again targeted women who were pregnant or nursing and young children, upholding the recommendation not to consume fish known to have high Hg concentrations, and added the suggestion that only 12 ounces (2 meals) of fish be consumed per week. According to Shimshack et al, the joint advisory was a rare response by the FDA, as the agency does not often issue such broad and direct campaigns (Shimshack et al 2007).

The EPA reports that mercury contamination is the leading cause of consumption advisories in the United States (80%) and Canada (97%). In 2006, 48 states had issued nearly 3,100 fish consumption advisories (Chalmers et al 2003). Of all the mercury species found in the environment, methylmercury is of the greatest concern because of its widespread environmental presence and its effects on neurological development and function (Ginsberg & Toal 2000). Methylmercury accounts for more than 95% of the mercury found in fish muscle (Sandheinrich et al 2011).

II. STATISTICAL ANALYSIS AND RISK ASSESSMENT

2.1 INTRODUCTION

2.1.1 Brief overview of risk assessments

Risk assessments seek to identify potential hazards to human health and estimate the nature and likelihood of adverse effects (US EPA 2012). The EPA lists 4 steps (Fig. 3) in completing a risk assessment: hazard identification, dose-response assessment, exposure assessment, and finally risk characterization.

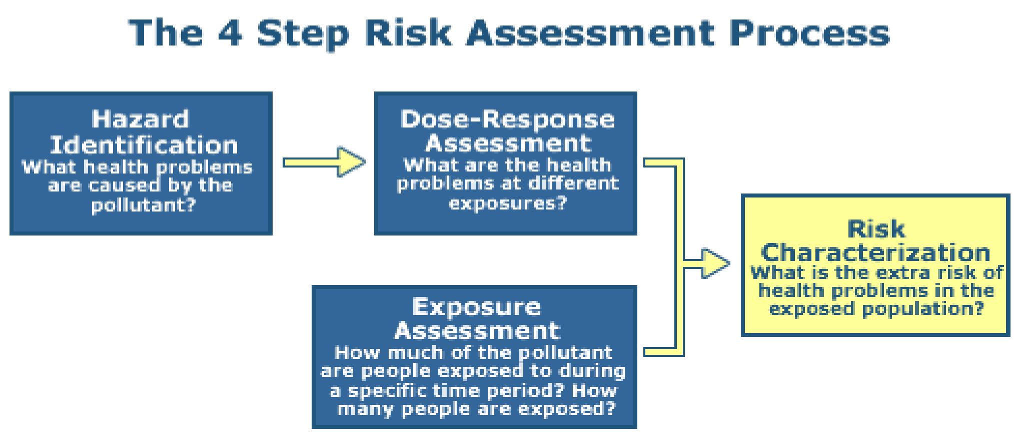


Figure 3: Steps in completing a risk assessment for hazards to human health

Hazard identification involves determining if exposure to a compound can cause an increase in adverse health effects and if those health effects can occur in humans. The data used to identify hazards usually comes from human clinical and epidemiological studies or animal studies. The dose-response assessment determines the relationship between the likelihood and severity of toxic effects caused by exposure to the hazard. For most toxicants, the response increases as the dose or exposure time increases. Dose-response curves vary with the compound and effect being measured. Exposure assessment seeks to determine the frequency, length, and degree of a person's exposure to a hazard. Both exposure pathway, how the hazard travels from its original source to the person, and exposure route, means by which the pathogen enters the body (absorption, inhalation, injection, ingestion) can affect how a compound interacts with the body and are considered when completing exposure assessments. Finally, the overall risk can be characterized. This step summarizes all the information gathered from the previous parts of the risk assessment to form general conclusions about the risk.

2.1.2 Importance of mercury risk assessments

Mercury is one of the most prevalent trace metal contaminants in the human food chain. It is released into the atmosphere via various natural and anthropogenic processes and it cycles through its different species by a series of complex biogeochemical processes. It is transferred to aquatic environments by wet and dry deposition and there it can be methylated to its organometallic form: MeHg. MeHg readily bioaccumulates and biomagnifies within the aquatic food web. Because it has a long half-life in fish, MeHg is usually found at the highest levels in predatory fish, with concentrations increasing as size and age increases.

The EPA attributes consumption of contaminated fish as the only source of MeHg for humans. In contaminated fish, MeHg accounts for 95% of the mercury in the muscle tissue (Sandheinrich et al 2011). MeHg is of particular concern because it is a neurotoxin that can cross the blood-brain barrier concern (UNEP 2008). Once consumed, nearly 100% of MeHg is absorbed from the gastrointestinal tract (Huggett et al 2001). It targets the brain and central nervous system as well as neural development in fetuses and children. In an attempt to protect consumers from the harmful effects of MeHg exposure, the FDA, EPA, and various Mississippi agencies routinely measure mercury concentrations in air, water, and fish and enforce regulations concerning acceptable Hg levels. The FDA has the least conservative threshold for mercury concentrations (1 µg/g), while the MDEQ enforces a more protective limit of 0.75 µg/g for fish tissue and 0.153 µg/L for water. See Table 1 for a listing of selected Hg concentration thresholds enforced by the EPA, FDA, and MDEQ.

Table 1: Selected federal and state mercury regulations currently in effect in Mississippi.

	Source	Hg Species	Maximum Concentration	Issuing Agency
Water	Drinking Water	Hg	2 ng/g	US EPA
	Freshwater Water Quality	Hg	770 ng/L	US EPA
	Freshwater Aquatic Life Support	Hg^{2+}	12 ng/L	MDEQ
	Marine Aquatic Life Support	Hg^{2+}	25 ng/L	MDEQ
	Drinking Water	Hg	≤ 151 ng/L	MDEQ
Fish	Consumption: Muscle	MeHg	300 ng/g	US EPA
	Consumption: Muscle	Hg	1000 ng/g	US FDA
	Consumption: Muscle	Hg	750 ng/g	MDEQ
	Water Quality: Whole Body	Hg	150 ng/L	MDEQ

Even though the regulations vary between agencies, they are often referenced when determining the risk associated with exposure to MeHg. The risk of health effects varies from person to person, depending on factors such as body weight, age, consumption frequency, and metabolism. Risk assessments make assumptions about the general public in order to evaluate the likelihood of negative effects associated with exposure to potential hazards. Using total-Hg data collected during a previous study (Brown 2013) of 202 fish from Grenada, Sardis, and Enid Lakes, the present analysis sought to evaluate the effect of different assumptions on the mercury risk assessment.

2.2 Methods

2.2.1 Sites

Grenada, Sardis, and Enid Lakes are major flood control reservoirs in the Yazoo River Basin in Northern Mississippi (Fig. 4). Spanning nearly 34600 km^2 and draining 30 counties in the northwestern part of the state, the Yazoo River Basin is the largest in the state. Grenada, Enid, and Sardis lakes have surface areas of 142 km^2, 131.5 km^2, and 84.5 km^2, respectively. The water levels of the three lakes are regulated throughout the year to help control flooding in the area. Levels are lowest in the drier months of the fall and winter and highest during the spring and summer months (MDEQ 2007).

The lakes are designated for recreational use, making them potential fishing locations for the general public and areas of concern for the Mississippi Department of Health. Grenada and Enid are currently under fish consumption advisories due to elevated mercury concentrations. While the MDEQ has not yet issued an advisory for Sardis Lake, the mercury concentration in fish is higher than the Mississippi guideline of

0.75ppm; however, under the FDA guideline of 1.0 ppm Sardis Lake is not considered impaired for mercury concentrations (Huggett et al 2001; MDEQ 2007).

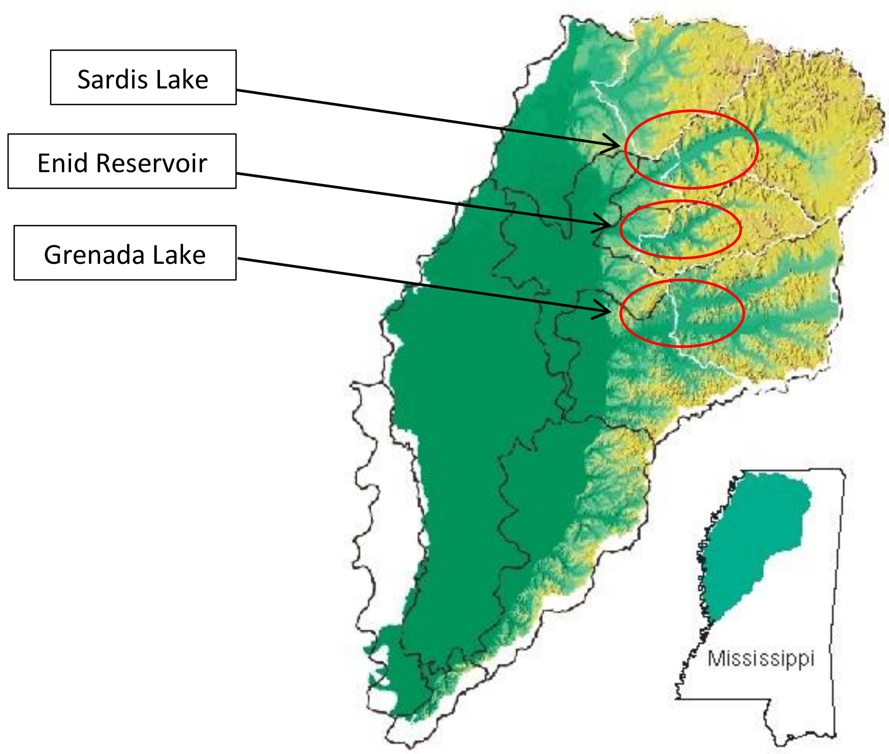


Figure 4: Map of the Yazoo River Basin showing the location of the three lakes from which fish were collected for the statistical analysis and risk assessment (USGS). The smaller map in the bottom right shows the location of the Yazoo River Basin (green) in Northwestern Mississippi.

2.2.2 Sampling

The fish collected from each lake were of species commonly sought after and consumed by local fishermen (See Table 2). Fish from Grenada and Sardis Lakes were caught by the Mississippi Department of Wildlife, Fisheries, and Parks (MDWFP) and samples from Enid Lake were collected by the USDA National Sediment Laboratory (NSL). A total of 202 fish of six different species were caught.

Electro-shocking is a non-lethal survey method used to temporarily paralyze the fish so they can easily be collected. Electrodes deliver 3-4 amps, depending on the lake's conductivity. The electrical field produced spans roughly 12 ft from the boat and penetrates 6-8 ft below the surface. Collection sites in Grenada Lake were near Young's Grenada, Bryant, and Gums Crossing Landings. In Sardis Lake fish were collected at the Hurricane Creek and Teckville Landings. At Enid Lake fish were collected from Cossar State Park.

Table 2: Number and species of fish collected from each lake

Species	Sardis	Grenada	Enid
Crappie (*Pomoxis annularis*)	20	20	16
Largemouth Bass (*Micropterus salmoides*)	21	18	15
Channel Catfish (*Ictalurus punctatus*)	18	14	15
Blue Catfish (*Ictalurus furcatus*)	4	6	--
Flathead Catfish (*Pylodictis olivaris*)	0	2	--
Gizzard Shad (*Dorosoma cepedianum*)	21	12	--
TOTAL	**84**	**72**	**46**

Once collected, the fish were placed on ice and taken to the University of Mississippi for analysis. In the laboratory the fish were dissected. The muscle and liver tissues were used for total-Hg analysis, while other organs including gills, gonad, kidney, heart, sperm, and eggs were preserved for use in other analyses. All samples were stored in individual vials and bags and frozen until analyzed (Brown 2013). For the present study, only data for the muscle for crappie, largemouth bass, and channel catfish were used.

2.2.3 Determination of mercury concentrations

Brown followed US EPA Method 7473 and measured the total-Hg concentration of the fish by direct mercury analysis using a Milestone DMA-80. The DMA-80 determined total-Hg concentration via thermal decomposition. Hg is released as the samples are heated and Hg vapor is trapped on a gold amalgamator before being desorbed and measured by atomic absorption spectrophotometry at 254 nm (Milestone, Inc. 2014).

Samples of fish muscle (0.2 g) were placed in nickel boats for analysis (Brown 2013). The DMA had a detection limit of 0.01 ng of Hg. Brown calibrated the instrument using liquid Hg standards and used DOLT-2 Certified Reference Material (Dogfish liver tissue) after every tenth sample to ensure the instrument was still working properly (Brown 2013).

2.2.4 Risk assessment calculations

Exposure to MeHg via fish consumption was estimated using methods outlined by the EPA (Huggett et al 2001). The risk assessment includes calculations of intake rate, hazard index (HI), monthly consumption limit (CR_{mm}) for both adults and children. The equations used are as follows:

$$\text{Intake Rate (mg kg}^{-1}\text{ day}^{-1}) = \frac{CF \times IR \times EF \times ED}{BW \times AT},$$

where CF is the mercury concentration in fish (mg kg^{-1}), IR is ingestion rate (kg meal^{-1}), EF is exposure frequency (meals year^{-1}), ED is exposure duration (year), BW is body weight (kg), and AT is averaging time (ED x 365 days year^{-1})

$$\text{HI} = \frac{intake\ rate}{RfD},$$

where intake rate is calculated using the previous equation and RfD is the reference dose for MeHg ($1\text{x}10^{-4}$ mg kg^{-1} day^{-1}). The EPA defines a reference dose as an estimated exposure dose at which no toxic effects will be suffered, even if the exposure is long-lasting. A hazard index is a ratio of an individual's actual exposure over a time period (here, 30 years) to the reference dose established by the EPA. While HI < 1, the expected potential for toxicity is low, and the exposure is considered safe. When HI > 1, there is an elevated potential for toxicity associated with the exposure. Once HI is calculated, monthly consumption limits are calculated. These consumption limits are often the basis for consumption advisories released by the EPA, FDA, and state agencies such as MDEQ.

$$CR_{mm} = \frac{RfD \times BW}{C_m} \times \frac{30.44 \frac{days}{month}}{IR},$$

where RfD is the reference dose ($1\text{x}10^{-4}$ mg kg^{-1} day^{-1}), BW is body weight, C_m is concentration in fish, an IR is ingestion rate.

For each calculation certain assumptions must be made about the body weight, ingestion rate, consumption frequency, and average meal size of the targeted population. The calculations were made using the mercury concentrations in crappie (CR), largemouth bass (LMB), and channel catfish (CC) collected in Grenada, Sardis, and Enid Lakes.

Seven different assumptions were tested in the risk assessment calculations. The assumptions used by Huggett et al. served as a "standard." Table 3 summarizes the assumptions made for each set of calculations.

Table 3: Values used for risk assessment calculations (IR = intake rate; BW = body weight; EF = exposure frequency; ED = exposure duration. The assumption that was varied for each set of calculations is listed in red.

Source	**IR** (kg meal^{-1})	**BW** (kg) Adult(Child)	**EF** (meals year^{-1})	**ED** (year)
Huggett et al.	0.227	70(14.5)	48	30
NOAA 2011	0.149	70(14.5)	48	30
American Heart Association	0.085	70(14.5)	48	30
MDEQ Consumption Advisory	0.227	70(14.5)	24	30
Portier (2007)	0.227	75(17)	48	30
EPA Exposure Factor Handbook	0.227	80(16)	48	30
NOAA 2011 & EPA Exposure Factor Handbook	0.142	80(16)	48	30

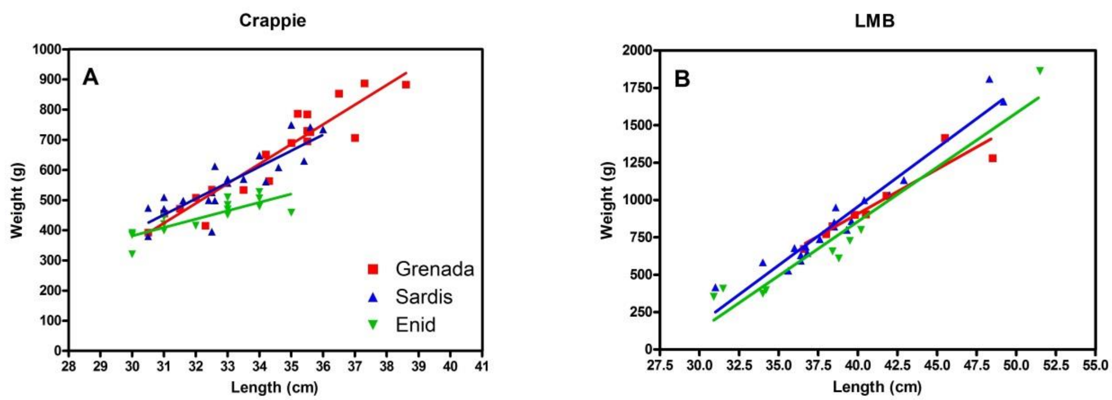
Statistical analysis and graphing of results was done using Microsoft Excel and GraphPad Prisim 4.0 software. We used the software for linear regression and ANOVA analyses to determine the relationship between mercury concentrations, fish weight, and fish length.

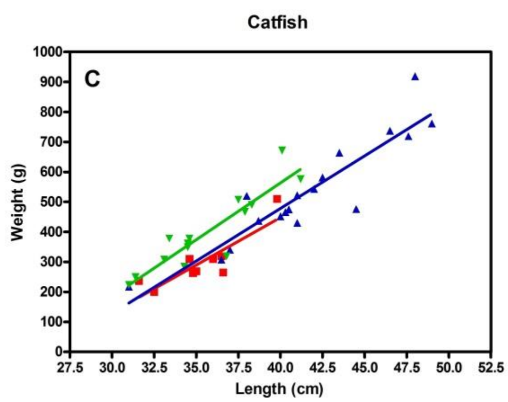
2.3 RESULTS AND DISCUSSION

2.3.1 Fish weight

Linear regression analysis showed a very strong direct relationship between fish weight and length for the species sampled in all three lakes (Fig. 5). For largemouth bass and catfish, the slopes for each lake did not differ significantly ($p = 0.242$ and $p = 0.8315$, respectively), suggesting that these species experience similar growth patterns regardless of lake.

The linear regression analysis of length v. weight slopes for crappie from Grenada and Sardis yielded a p value of 0.2003, indicating that the difference between the two slopes was not significantly different. Therefore, crappie in Grenada and Sardis Lakes likely exhibit similar growth trends. However, crappie from Enid had a length vs. weight slope that was significantly different from both Grenada ($p = 0.00017$) and Sardis ($p = 0.01037$), suggesting that water quality and other environmental factors unique to Enid Lake may affect the growth of crappie.





	r^2			p-value
	Grenada	Sardis	Enid	
CR	0.8820	0.7386	0.7063	0.0006
LMB	0.8835	0.9417	0.9259	0.242
CC	0.7442	0.8359	0.8248	0.8315

Figure 5: Linear regression analyses of fish length vs. fish weight data from Grenada, Sardis, and Enid Lakes. The table lists the r^2 values for each line as well as the p values from a linear regression analysis of the three lines on each graph. Red p-values indicate a statistical significance in the differences of the slopes.

2.3.2 Fish mercury concentration

Of the three fish species collected from each lake, LMB consistently had the highest average Hg concentrations (Grenada: 630 ± 104 ng/g, Sardis: 334 ± 40 ng/g, Enid: 386 ± 36 ng/g). The average Hg concentrations in LMB for all three lakes exceeded the threshold concentration of 300 ng/g that is enforced by the EPA. Grenada Lake produced LMB and CC with the highest average concentrations (630 ± 102 ng/g and 437 ± 42 ng/g, respectively), followed by Sardis (334 ± 40 ng/g and 222 ± 21 ng/g) and then Enid (386 ± 76 ng/g and 152 ± 14 ng/g). Crappie from Enid had the highest average Hg concentration (214 ± 10 ng/g) while CR from Sardis had the lowest average concentration (147 ± 8 ng/g).

Table 4: Average mercury concentrations (ng/g), standard errors (1 SE), minimum, maximum, and median concentrations for CR, LMB, and CC collected from Grenada, Sardis, and Enid Lakes. Values in red represent Hg concentrations that exceed the maximum concentrations allowed by the EPA (300 ng/g). The value in blue exceeds the maximum concentration allowed by the MDEQ (750 ng/g). The value in green exceeds the maximum concentration allowed by the FDA (1000 ng/g)

	Grenada			Sardis			Enid		
Hg (ng/g)	CR n = 20	LMB n = 8	CC n = 9	CR n = 20	LMB n = 19	CC n = 18	CR n = 16	LMB n = 9	CC n = 14
Average	199	630	437	147	334	222	214	386	152
1 SE	17	104	42	8	40	21	10	76	14
min	99	351	261	109	102	142	120	184	84
max	383	1066	666	237	723	432	285	954	272
median	180	541	440	138	279	190	215	344	146

Figure 6 compares the mean Hg concentrations of the fish collected from each lake. A one-way ANOVA and a post hoc test was used to determine if the concentrations were statistically different from one another. LMB Hg concentrations did not differ significantly between the three lakes, but they were statistically higher than crappie from Sardis and catfish from Enid. Similarly, the Hg concentrations in crappie did not differ significantly between lakes. The mean Hg concentration in catfish from Grenada Lake was statistically higher than that in catfish from Sardis and Enid. Hg concentrations in LMB from Grenada Lake were statistically higher than crappie from Grenada and Sardis as well as catfish from Sardis and Enid.

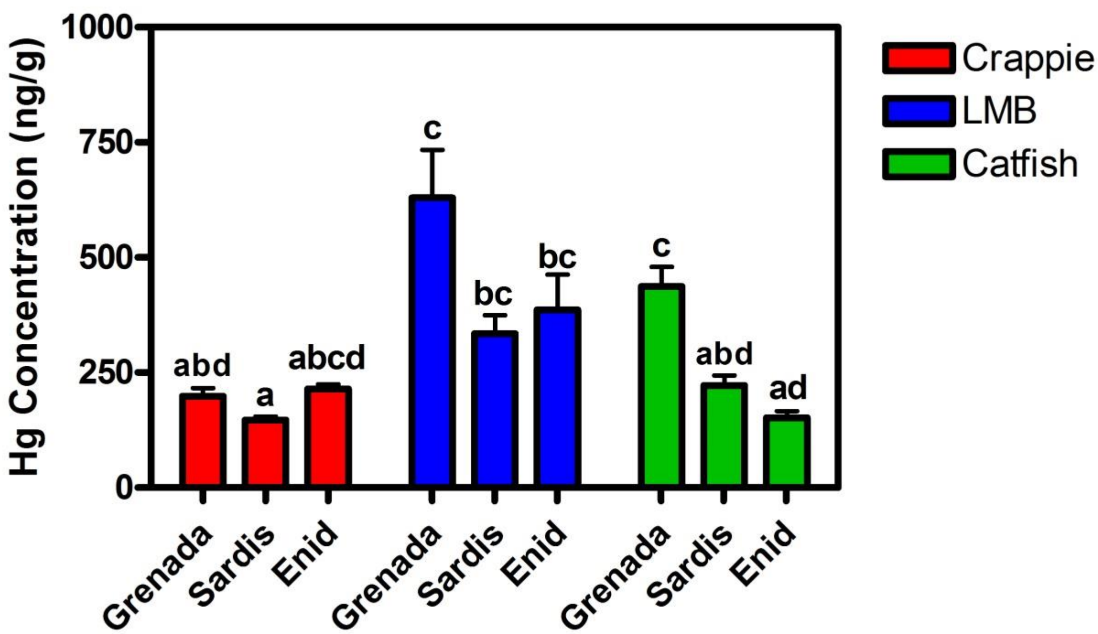


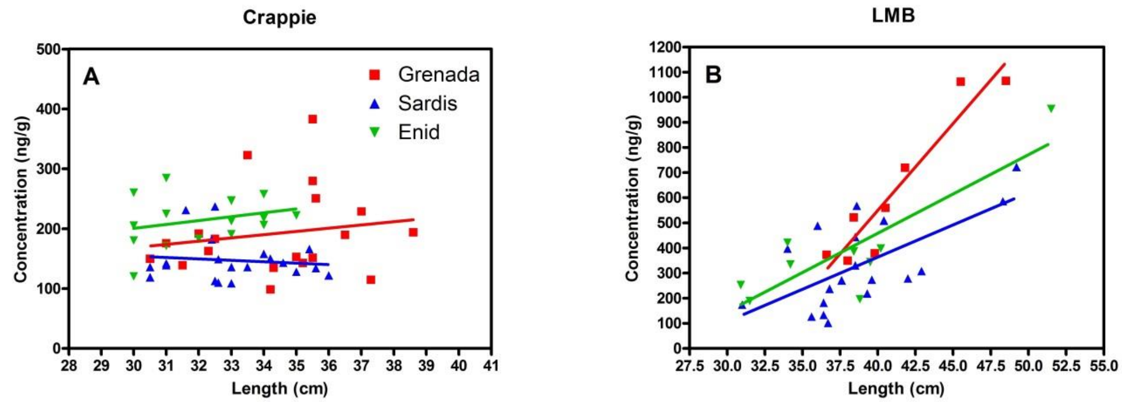
Figure 6: Comparison of mean ± standard error Hg concentration and standard error of fish collected from each lake. The letters above each bar indicate statistical difference of the average Hg concentration. Bars that share a letter are not significantly different from one another.

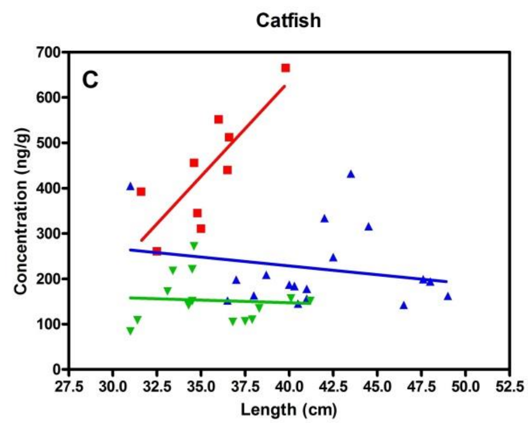
In 1999 Hugget et al also found that Hg concentrations in LMB (1400 ng/g) and CR (1690 ng/g) from Enid Reservoir were higher than in CC (820 ng/g). While our measurements follow a similar trend, they also show that overall Hg concentrations in

Grenada, Sardis, and Enid Lakes have decreased since Hugget's study, likely as a result of MDEQ recently refocused efforts to locate possible sources of mercury and monitor water quality (MDEQ 2012).

2.3.3 Fish length vs. Hg concentration

Length can reflect the age of the fish and, because older fish tend to have higher Hg concentrations, it can sometimes provide a general idea about the extent of Hg bioaccumulation in its tissues. Many risk assessments and the resulting consumption advisories assume a direct relationship between fish length and mercury concentration, often using length as an indicator of the risk of Hg exposure. However, factors other than length, such as diet and metabolism, can also affect the bioaccumulation of mercury in fish. Our data suggests that not all the species tested (CR, LMB, and CC) follow the expected trend.





		Grenada	Sardis	Enid
CR	r^2	0.029	0.013	0.075
	p-value	0.4883	0.6336	0.3060
LMB	r^2	0.895	0.413	0.775
	p-value	0.0004	0.0030	0.0036
CC	r^2	0.6307	0.0396	0.0048
	p-value	0.0106	0.4285	0.8140

Figure 7: Linear regression analyses of fish length vs. Hg concentration data from Grenada, Sardis, and Enid Lakes. The table lists the r^2 values for each line as well as the p values from a linear regression analysis of the three lines on each graph. Red p-values indicate a statistical significance in the relationship between fish length and fish weight.

LMB was the only species that showed a steady increase in Hg concentration as fish length increased in all three lakes. Liner regression analyses of length vs Hg concentrations for LMB from Grenada, Sardis, and Enid Lakes yielded the most direct relationship between length and concentrations, with positive slopes and r^2 values of 0.8951, 0.4131, and 0.7247, respectively. The small p-values for LMB indicate a significant relationship between length and Hg concentration at all three lakes.

Largemouth bass are large freshwater fish that are higher in the food chain. The average length of LMB in the data set used for this analysis was Grenada: 41 ± 1 cm, Sardis: 39 ± 1 cm, Enid: 38 ± 2 cm. LMB prey on smaller fish, increasing the likelihood that mercury will bioaccumulate in their tissues (ADCNR nd).

Crappie collected from each lake showed no relationship between fish length and mercury concentration. Linear regression analysis yielded r^2 that were extremely close to zero and large p-values, suggesting that there is no relationship between length and Hg concentration in crappie. Crappie are smaller fish that typically feed on small minnows and insects, which would be expected to have low mercury concentrations (TWPD nd). Because they are smaller fish and occupy a lower trophic lever in the aquatic ecosystem, crappie are not likely exposed to high Hg concentrations through their diet. As reflected by the lower average Hg concentrations, they may also be more efficient at metabolizing and then excreting mercury than LMB and CC.

In catfish the relationship between length and concentrations varied by lake. Samples from Grenada had a positive slope and the highest r^2 value (0.6307), indicating that there might be a weak relationship between length and Hg concentrations. The small p-value for CC at Grenada Samples from Sardis and Enid had slightly negative slopes and very small r^2 values. The relationships between length and Hg concentration in CC from Sardis and Enid were not statistically significant.

The diet of channel catfish consists of insect larvae, small mollusks and fish and some plant material, all of which likely have low Hg concentrations (ADCNR nd). Catfish are bottom dwellers and may be exposed to mercury in sediment (TWPD nd).

Measurements of the Hg concentration in sediment from Grenada, Sardis, and Enid in 1999 by Huggett et al showed that Grenada had the highest concentration (39 ng/g) followed by Enid (34 ng/g) and Sardis (19 ng/g). The differences in the sediment Hg concentrations of the lakes may account for some of the variation in CC Hg concentrations.

As discussed in Section 2.4.1, catfish and largemouth bass show similar growth trends in all three lakes. However, the catfish from the three lakes showed varying relationships length vs. Hg concentration. This may suggest differing mercury concentrations in Grenada, Sardis, and Enid Lakes. Catfish from Grenada had the steepest slope for length v. Hg concentration, indicating that Grenada Lake may have a higher Hg concentration than the other two lakes. The slopes of length vs Hg concentration for LMB from Grenada and crappie from Grenada were also steeper than those for Sardis and Enid.

The MDEQ has yet to identify any point sources that may be directly contributing to the mercury contamination of the lakes (MDEQ 2007). The differing concentrations may be related to water chemistry such as temperature, dissolved oxygen content, total dissolved solids, and pH. Because mercury can move between aquatic and terrestrial biospheres, water-soil interactions can also impact mercury concentrations in the individual lakes.

2.3.3 Risk assessment

Risk assessments are calculated to evaluate the hazard to adults and children associated with the consumption of contaminated fish. The assessments serve as a guide for issuing consumption advisories and making suggestions about safe consumption limits. Because risk assessments require making assumptions about the body weight and consumption habits of the population, the EPA recommends selecting values that are most relevant to the specific audience, including the type of fish sampled and the age group targeted (EPA, Intake of fish and shellfish 2011).

We completed the risk assessment calculations for CR, LMB, and CC from Grenada, Sardis, and Enid Lakes a total of seven times, varying at least one assumption value each time. The equations and values used are listed and explained in Section 2.3.4 and Table 3.

The assumptions used by Huggett et al came from the EPA and served as a starting point for our calculations and analysis. Using an ingestion rate of 0.227 kg $meal^{-1}$ (8 oz), adult and child body weights of 70 kg and 14.5 kg, respectively, and an exposure frequency of 48 meals $year^{-1}$ (4 meals per month), the assessment based on Huggett's values proved the least conservative. The most conservative assessment used the American Heart Association's recommendation of 3 ounce (0.085 kg) servings of fish.

We also varied the body weight values for the risk assessment. Since Hugget's 1999 study was published, the average body weights for adults and children have increased. The 2011 EPA Exposure Handbook listed the results of more recent body weight studies, reporting an average body weight of 80 kg for adults and 16 kg for children. While using the higher body weights did result in a lower calculated hazard index and slightly higher MCLs, the overall conclusion for the risk assessment did not change.

In all our variations of the risk assessment, LMB from Grenada Lake had a HI>1 indicating an increased risk for toxic effects. Similarly, only the assessment that assumed an average consumption of 15 lbs per person per year (NOAA 2011), which equals roughly 5.25 oz four times a month, yielded a HI<1 for CC from Grenada. Based on our calculations, crappie was the only fish consistently safe to consume from all three lakes. None of the fish sampled yielded a HI<1 for children in any of the risk assessments.

Due to the adverse neurological and developmental effects associated with mercury toxicity, many consumption recommendations target children and pregnant women. The EPA, FDA, and AHA suggest these groups limit fish intake to 12 oz (2 meals) per week (6 oz/meal, 8 meals/month, 108 meals/year) in order minimize the risk of mercury contamination.

Of the risk assessment paradigms tested in this study, NOAA's estimation of 15 lbs per person per year (approximately 5.25 oz/meal, 48 meals/year) is the closest ingestion rate to the 12 oz/week recommendation issued by the FDA. Based on the results of the risk assessment, the 12 oz recommendation is not safe for children for any of the fish collected. For adults it is safe to eat crappie from Grenada, all three species from Sardis, and crappie and catfish from Enid. Table 5 lists the calculated hazard indices and monthly consumption limits for adults and children from each of the risk assessments performed. Most of the calculated MCLs for adults were well below 8 meals/month. The FDA's 12 oz recommendation is not protective enough for the general public. Advisories for at risk populations, such as children and pregnant women, should be more conservative in order to minimize exposure to mercury.

In the risk assessments, we also calculated the monthly consumption limit (MCL) in meals/month. The MCL is the number of meals it is safe for adults and children to consume based on the fish Hg concentration and assumptions made for the calculations. For Hugget's risk assessment, all of the MCLs for adults were below the AHA's recommended 8 meals/month and many were below the assumed 4 meals/month. Only CR from Grenada, CR and CC from Sardis, and CR and CC from Enid had MCLs of 4 or greater for adults.

In assessments using smaller serving sizes, the MCL for adults was much closer to the assumed 4 meals and the recommended 8 meals. Using NOAA's reported 15lbs/person/year (5.25 oz/meal), only LMB from all three lakes, and CC from Grenada fell below the assumed 4 meals/month. However, only CR from Grenada and Sardis and CC from Enid had MCLs above 8 meals/month. Using the most conservative ingestion rate of 3 oz, all the adult MCLs were above 4 meals/month and all except LMB and CC from Grenada were at least 8 meals/month. For children the calculated MCL was 2.5 or below for all of the risk assessments completed.

The MDEQ has issued a fish consumption advisory warning the public not to consume more than 2 meals/month (24 meals/year) of CC larger than 27 inches. However, they have not placed a monthly limit on LMB or CR consumption. When 24 meals/year was used for the consumption frequency and 8 oz of fish/meal in the risk assessment calculations, only LMB from Grenada Lake had an HI>1.

Crappie from Grenada, Sardis, and Enid Lakes yielded adult MCLs of 5.0, 6.5, and 4.5 respectively. Though Hg concentrations in CR may not necessitate an advisory as strict as 2 meals/month issued for CC, adults should not eat more than 5 or 6 meals of crappie in a single month in order to limit exposure to MeHg. Similarly, there should be a limit on how many meals of LMB adults are advised to consume. Grenada LMB had an MCL of less than 2 and LMB from Sardis and Enid had MCLs of 3.5 and 3, respectively. Channel catfish from the lakes had adult MCLs of 2.0, 4.5, and 6.5 meals/month; therefore, the MDEQ's 2 meals/month consumption advisory is sufficient for adult consumption of catfish from all three lakes.

Table 5: Comparison of Mean HI (Hazard Index) and MCL (Mean Consumption Limit in meals/month) for each set of risk assessment assumptions. HI values in red are above the EPA's current recommended threshold of HI = 1. See Table 3 in Section 2.3.4 for complete listing of assumptions made for each set of risk assessment calculations. MCL values are conservatively rounded down to the nearest 0.5 meals.

		Hugget				Ingestion Rate (15lbs/person/year)				Ingestion Rate (3 oz/meal)				Consumption Frequency			
		Mean HI		MCL		Mean HI		MCL		Mean HI		MCL		Mean HI		MCL	
Lake	Species	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child
Grenada	CR	0.85	4.11	5.0	1	0.53	2.56	8.5	1.5	0.32	1.53	14.0	2.5	0.42	2.05	5.0	1.0
	LMB	1.56	7.55	3.0	0.5	1.68	8.11	2.5	0.5	1.01	4.85	4.5	0.5	1.34	6.48	1.5	0.0
	CC	1.81	8.75	2.0	0.5	1.17	5.63	3.5	0.5	0.70	3.37	6.0	1.0	0.93	4.50	2.0	0.0
Sardis	CR	0.63	3.02	6.0	1	0.392	1.89	10.5	2.0	0.235	1.13	17.5	3.5	0.313	1.51	6.5	1.0
	LMB	1.42	6.88	3.0	0.5	0.892	4.31	6.0	1.0	0.534	2.58	10.0	2.0	0.713	3.44	3.5	0.5
	CC	0.95	4.58	4.0	1	0.593	2.86	7.5	1.5	0.355	1.71	12.5	2.50	0.474	2.29	4.50	0.50
Enid	CR	0.91	4.41	4.0	1	0.572	2.76	7.0	1.5	0.342	1.65	12.0	2.50	0.457	2.21	4.50	0.50
	LMB	1.65	7.96	3.0	0.5	1.031	4.98	4.5	0.5	0.617	2.98	8.0	1.50	0.824	3.98	2.50	0.50
	CC	0.65	3.14	7.0	1	0.406	1.96	10.5	2.0	0.243	1.17	18.0	3.50	0.325	1.57	6.50	1.00

Table 5: (CONTINUED): Comparison of Mean HI (Hazard Index) and MCL (Mean Consumption Limit in meals/month) for each set of risk assessment assumptions. HI values in red are above the EPA's current recommended threshold of HI = 1. See Table 3 in Section 2.3.4 for complete listing of assumptions made for each set of risk assessment calculations. MCL values are conservatively rounded down to the nearest 0.5 meals.

		Body Weight (Portier 2007)				Body Weight (EPA 2011)				Ingestion Rate & Body Weight (EPA 2001)			
Lake	Species	Mean HI		MCL		Mean HI		MCL		Mean HI		MCL	
		Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child
Grenada	CR	0.79	3.49	5.5	1.0	0.74	3.72	6.0	1.0	0.46	2.32	9.5	2.0
	LMB	2.51	11.0	1.5	0.0	2.35	11.0	2.0	0.0	1.47	6.91	3.0	0.5
	CC	1.74	9.01	2.0	0.5	1.63	8.16	2.0	0.5	1.02	5.11	4.0	0.5
Sardis	CR	0.585	2.58	7.0	1.5	0.548	2.74	7.5	1.5	0.343	1.71	12.0	2.0
	LMB	1.331	5.87	4.0	0.5	1.248	6.24	4.0	0.5	0.780	3.90	6.5	1.0
	CC	0.885	3.90	5.0	1.0	0.830	4.15	5.0	1.0	0.519	2.60	8.5	1.5
Enid	CR	0.853	3.76	4.5	1.0	0.800	4.00	5.0	1.0	0.800	4.00	8.0	1.5
	LMB	1.538	6.79	3.0	0.5	1.442	7.21	3.0	0.5	0.902	4.51	5.0	1.0
	CC	0.606	2.67	7.0	1.5	0.568	2.84	7.5	1.5	0.356	1.78	12.0	2.0

2.3.5 Discussion of existing consumption advisories

The current fish consumption advisories for Grenada and Enid lakes warn locals not to eat largemouth bass or catfish longer than 27 inches (68.58 cm). The existing advisories do not place a length limit on crappie. The advisories are in place to protect consumers from fish that are most likely to have the highest MeHg concentrations. The EPA warns that a calculated hazard index (HI) of greater than 1 indicates an increased potential for toxicity.

The MDWFP, concerned with protecting the crappie population, only enforces a minimum length of 11 in (27.94 cm) on crappie caught at any of the lakes. The average length for crappie collected and analyzed for this study is 33 cm (12.99 in). The smallest crappie with an HI>1 from each lake was Grenada: 33.6 cm, Sardis: 32.5 cm, and Enid: 33.0 cm.

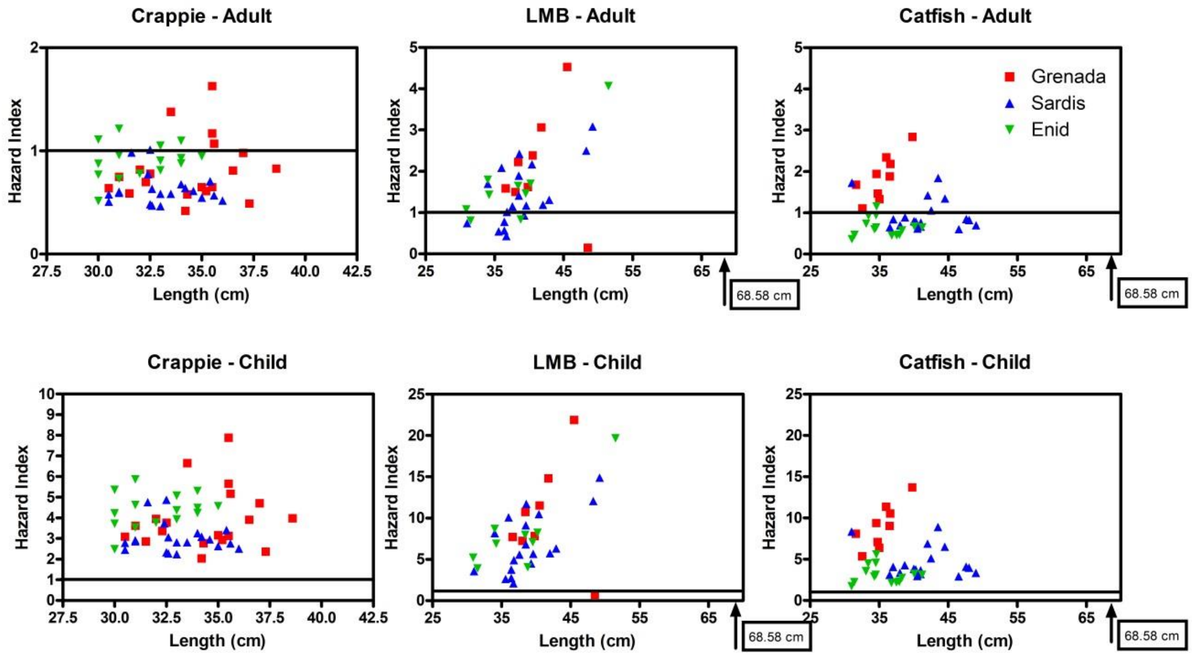


Figure 8: Fish length vs. Hazard Index (calculated using assumptions in Huggett et al) for Crappie, LMB and Catfish. The arrow below the x-axis of the graphs for LMB and CC indicates 27 inches (the current MDEQ fish consumption advisory for CC from Grenada and Enid) compared to the fish collected.

Figure 8 shows length vs. HI (calculated using Huggett's assumptions) for all the fish analyzed in this study. All of the LMB and CC collected and analyzed in this study were well below the 27" guideline issued by the MDEQ. However, the calculated adult HI for many of the fish was much greater than 1. For LMB from Grenada Lake (n = 8), 7 fish had an HI > 1 for adults. The results were similar for LMB collected from Sardis (n = 19) and Enid Lakes (n = 9). All of the catfish collected at Grenada Lake (n = 9) had an HI > 1. Five of the catfish from Sardis Lake (n = 18) had an HI > 1 for adults. Only 1 catfish from Enid Lake (n = 14) had a MeHg concentration high enough to yield an HI > 1 for adults.

Because nearly 100% of the LMB analyzed yielded an adult HI>1, the samples are likely representative of the entire LMB population in Grenada, Sardis, and Enid Lakes. Similarly, the CC collected from Grenada and Enid were representative of the entire population. Nearly all of the fish collected, regardless of species or lake, had a child HI well above 1.

The length-based consumption advisories issued by the MDEQ for LMB and CC from Grenada and Enid Lakes are not conservative enough to protect the general public from exposure to MeHg. Figure 6 in Section 2.4.1 indicates a statistical difference in the Hg concentrations of LMB from Enid and Grenada and the CC from Enid. Thus, the advisory may be a useful rule for CC caught at Enid Lake, but remains insufficient for LMB caught at Enid and Grenada Lakes as well as CC from Grenada Lake. As described in Section 2.4.3, crappie and channel catfish do not exhibit a strong direct relationship between length and MeHg concentration. Therefore, a length-based consumption advisory for these species does not offer much protection from MeHg. Though the MDEQ has yet to issue a consumption advisory for Sardis Lake, many of the fish there had MeHg concentrations high enough to yield an HI > 1, indicating a significant potential for toxicity.

Many of the fish analyzed in this study also had Hg concentrations higher than the threshold values for muscle Hg concentration set by the EPA, FDA, and MDEQ (see Table 1 for regulatory values and Table 4 for Hg concentrations). The EPA threshold concentration is the most conservative at 0.3 µg/g. The average Hg concentration in LMB and CC from Grenada and LMB from Sardis exceeded this value. These same species yielded adult HI>1 using the Huggett assumptions for risk assessment calculations, suggesting that the EPA regulatory value is conservative enough to protect consumers from exposure to MeHg.

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